The Agena iPLEX HS Lung Panel on the MassARRAY System Is Able To Robustly Characterize the Molecular Profile of FFPE Derived Lung Tumor Samples Previously Deemed QNS on Multiple NGS Platforms

Alexander Sartori[1], Anurodh Agrawal[2], Alex Gonzalez[2], Katie Utt[2], Darryl Irwin[1], Brandon Franklin[1], Hannah Payne[2], Meredith Berry[2], Darren Davis[2]

[1] Agena Bioscience Inc., San Diego, CA, USA, [2] Precision For Medicine, Houston, TX, USA. Contact: Darren.Davis@precisionformedicine.com

Introduction

Somatic variant profiling of multiple genes simultaneously in cancer samples has become common practice, primarily as predictive biomarkers for targeted therapies.

Formalin-fixed, paraffin-embedded (FFPE) tissue is the gold standard method for preserving tissues for downstream analysis. However, extracting nucleic acid from FFPE tissues is challenging due to limited sample quantity, poor quality, low variant allele frequency (VAF) due to few tumor cells and low tumor nuclei percentage, as well as being fragmented and chemically modified.

These challenges can result in high quality control (QC/QNS) failure rates (up to approximately 45% for some tests) which prevents the generation of accurate results. The targeted Agena iPlex® HS Lung Panel on the MassARRAY® System (Figure 1) overcomes the challenges posed by limited quantity, low VAF, and poor-quality in FFPE samples.

Materials & Methods

196 lung tissue FFPE samples were identified that had previously been extracted using MagMAX FFPE DNA/RNA ultra kit and determined to be quality/quantity not sufficient (QNS) or failed sequencing on two NGS platforms (Figure 2): 89 samples with the TruSight Ocology 500 Assay (TSO 500; Illumina) and 107 samples with the Oncomine Precision Assays (OPA; ThermoFisher).

All samples were processed at Precision for Medicine on the MassARRAY® System with the Agena iPLEX® Pro Sample ID Panel for DNA integrity and the Agena iPLEX® HS Lung v2 Panel, a targeted molecular profiling panel that examines 89 hotspot variants across 5 genes (*KRAS, EGFR, BRAF, ERBB2 and PIK3CA*). The Panel incorporates a single global PCR reaction from ≤20ng FFPE tissue derived DNA with short amplicon PCR and mutation enriched single base extension chemistry.

Positive and Negative Predictive Values (PPV/NPV) were determined by a platform comparison to droplet digital PCR (ddPCR, Bio-Rad).

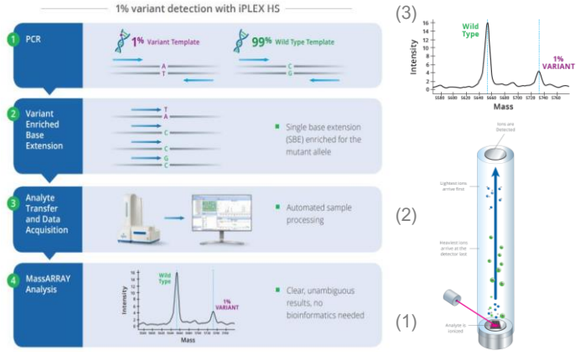

Figure 1: Left: Flow of the iPLEX® HS biochemistry with >1% LoD. (1) Single global PCR for amplicon generation, (2) Variant enriched multiplexed single base extension reaction, (3) Fully automated sample transfer and data acquisition on the MassARRAY® System, (4) automated data analysis and reporting. **Right: (1)** In the MassARRAY Analyzer, DNA analytes are irradiated by a laser, ionized (positively charged) and begin to migrate through the vacuum tube. (2) Based on their mass, the analyte ions arrive at the detector at different times (or times of flight). (3) The integrated software interprets the mass spectra and generates a user-friendly report.

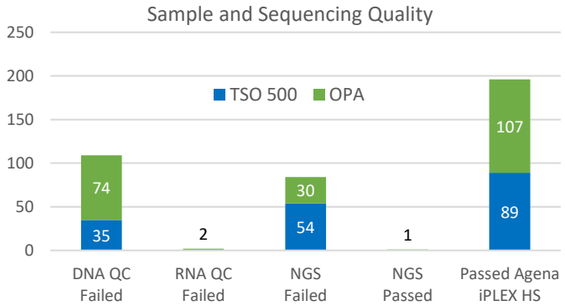

Figure 2: Of the 196 samples analyzed using the iPLEX HS Lung Panel, 109 and 84 were classified as "DNA-QC failed" and "DNA NGS failed", respectively; 2 samples were excluded from NGS although DNA-QC was passed (but RNA-QC failed); 1 sample was incorrectly labeled as "DNA-NGS failed". The retrospectively reported EGFR-L858R mutation was detected with MassARRAY.

Results

Molecular Analysis:

- All 196 samples passed the iPlex® Pro QC parameters.
- Using the iPLEX® HS Lung Panel, a total of 81 somatic mutations were detected in 74/196 (38%) of the samples (Table 1).
- The distribution of detected mutations was as follows: *KRAS* (n=45; 23% of samples), *EGFR* (27; 14%), *BRAF* (3; 2%), *ERBB2* (4; 2%) or *PIK3CA* (2; 1%).

Orthogonal Validation:

- Orthogonal validation was performed on 51/196 (26%) positive and 33 randomly selected negative samples with commercially available ddPCR *KRAS* G12/G13, *KRAS* Q61, *EGFR* Exon 19 Deletions, *EGFR* L858R, and *BRAF* V600 Screening Kits using QX200 ddPCR system (Bio-Rad).
- 100% (51/51) PPV and 99.7% (666/668) NPV was observed based on ddPCR result (Table 2).
- Two samples showed a 2% and 2.5% *KRAS* G12/G13 mutation by ddPCR but were initially not detected by the iPLEX® HS Lung panel.
- Though, manual inspection showed peaks indicating KRAS p.G12V and p.G13D mutation (Figure 3) that were not reported by the Somatic Variant Reporter software using the default analysis parameters.

iPLEX HS Lung Summary	#	%
Samples Total	196	100%
Samples with Mutations	74	38%
Mutations Total	**81**	
BRAF	3	2%
G469V	1	1%
V600E	2	1%
EGFR	27	14%
Exon 19 Del	8	4%
G719X	2	1%
L858R	11	6%
L861Q	1	1%
S768I	3	2%
T790M	2	1%
ERBB2 Insertions	4	2%
KRAS	45	23%
G12A	2	1%
G12C	22	11%
G12D	5	3%
G12R	1	1%
G12V	7	4%
G13C	3	2%
G13D	1	1%
Q61H	4	2%
PIK3CA	2	1%

Table 1: Breakdown of 81 mutations by gene and variant detected on the Mass-ARRAY in 74 of the 196 samples that had not previously been detected by NGS.

Summary of orthogonal validation using ddPCR mutation kits	Samples #	Mutations #	Concordance ddPCR and MassARRAY
HS Lung positive mutations	51	51	100.0%
BRAF V600 positive	1	1	100.0%
KRAS G12/13 positive	30	30	100.0%
KRAS Q61 positive	4	4	100.0%
EGFR L858R positive	9	9	100.0%
EGFR Exon 19 Del positive	7	7	100.0%
HS Lung negative mutations	33	666/668	99.7%
BRAF V600 negative	6	6/6	100.0%
KRAS G12/13 negative	21	325/327	99.4%
KRAS Q61 negative	19	111/111	100.0%
EGFR L858R negative	6	6/6	100.0%
EGFRExon 19 Del negative	8	218/218	100.0%

Table 2: For the 51 positive and 33 randomly selected negative samples, orthogonal validation using ddPCR resulted in 100% and 99.7% concordance, respectively.

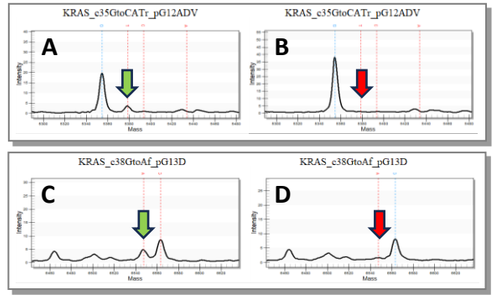

Figure 3: Specific peaks in the mass spectra indicate the presence of the missed KRAS G12V (A) and KRAS G13D (C) mutations (green arrows). For comparison, mass spectra B and D of samples negative for the respective mutations (red arrows).

Conclusions

- The Agena iPLEX® HS Lung Panel on the MassARRAY® System is highly tolerant of poor quantity and quality DNA.
- This technology is recovering and delivering accurate results on samples that would otherwise fail NGS-based analysis.

AMP 2024 Vancouver, BC – Poster ST143 – Abstract #1873892

Learn more at booth #925